

Development of clade-specific 16S rDNA oligonucleotides for use in assessing the spatial and temporal dynamics of community structure in the photosynthetic picoplankton genera *Synechococcus* and *Prochlorococcus*

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PICODIV

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Key issues

What controls marine primary productivity?

- requirement to study representative organisms

What factors contribute to their success in the marine environment?

- ability to sense and rapidly respond to environmental stimuli - how do they do this?
- adaptation to a niche?
- if so, what are the molecular and biochemical mechanisms of niche adaptation?

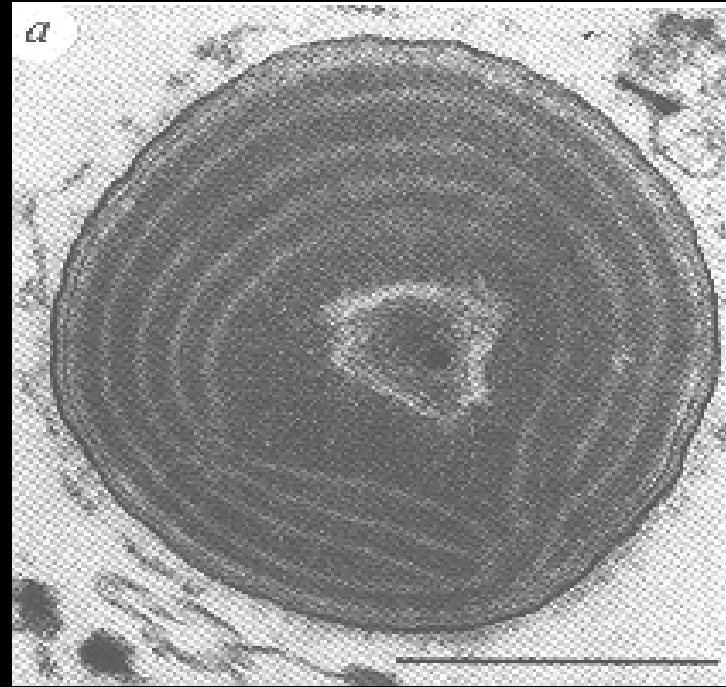
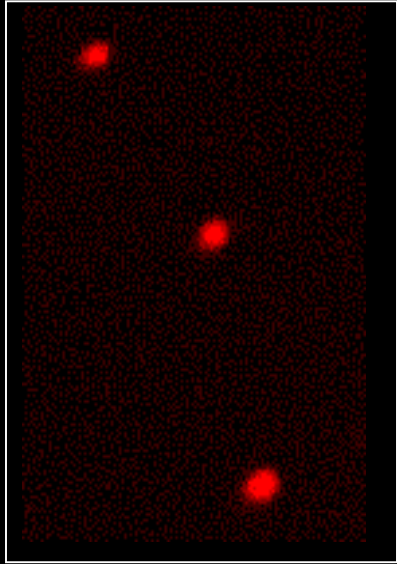
Aims

- Characterisation of dynamics of cyanobacterial community structure at the molecular level
- Correlation with physical & chemical properties of the water column and phenotypic analyses (PstS expression)

Questions:

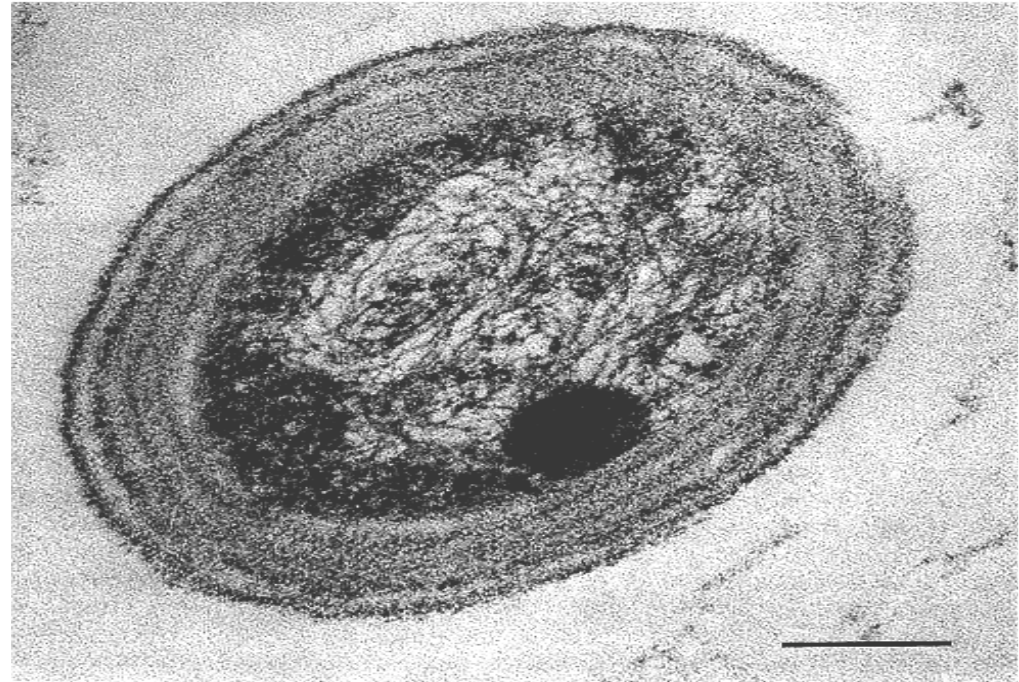
- How does the distribution of different genotypes vary temporally and spatially- do they occupy a specific niche?
- What factors affect this variation?
- What are the dominant genotypes?
- Are lab cultures representative of strains dominant *in situ*?
- Do different genotypes have different C-fixation capabilities?

Marine *Synechococcus*



- Small unicellular cyanobacterium (ca. 1 μm)
- Contain phycobilisomes
- Orange-yellow fluorescence under blue light
- Some motile strains
- Global distribution, throughout euphotic zone
- Up to 10^4 - 10^5 cells ml^{-1}

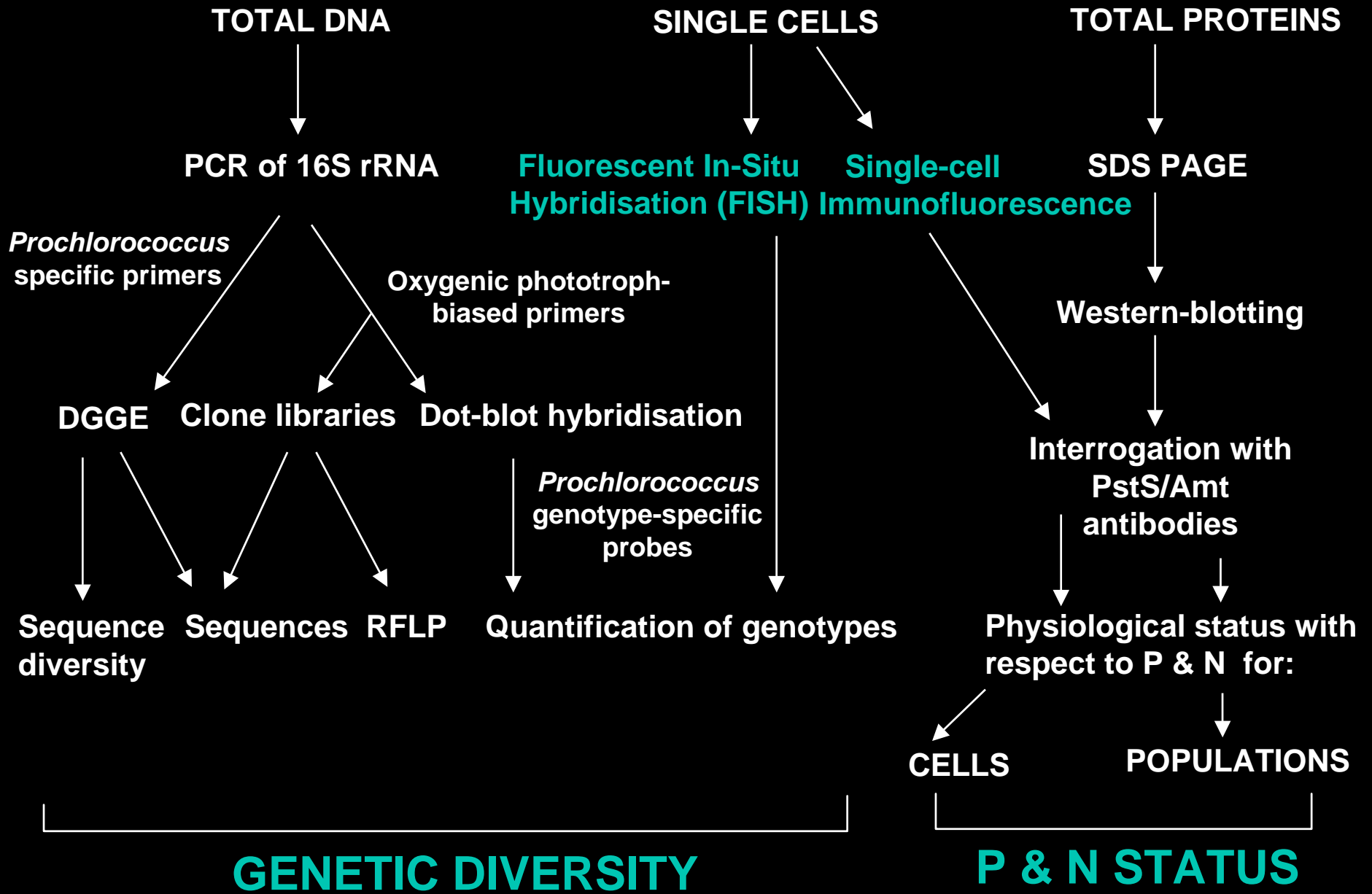
Prochlorococcus

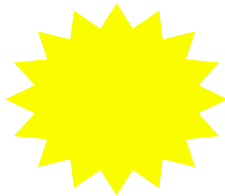


- small marine unicellular cyanobacterium (0.6 μm)
- no phycobilisomes
- divinyl-chlorophyll *a* and *b*
- red fluorescence under blue light

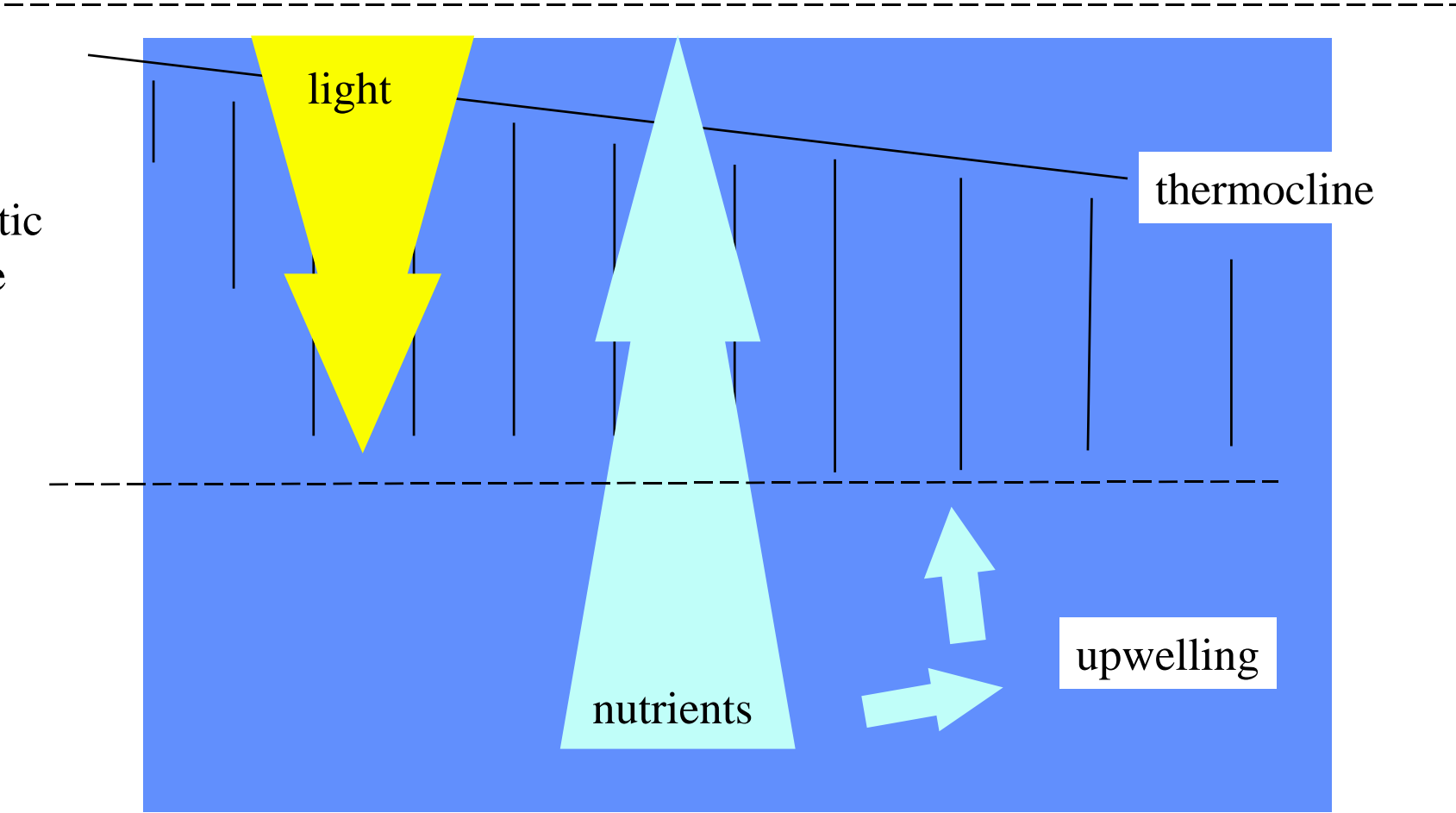
So, why are these organisms good choices for study?

- known to be important contributors to 1^o production
- easily enumerated *in situ* using flow cytometry
- readily isolated into culture
- genomic information is now available
- genetic system available for *Synechococcus*





euphotic zone

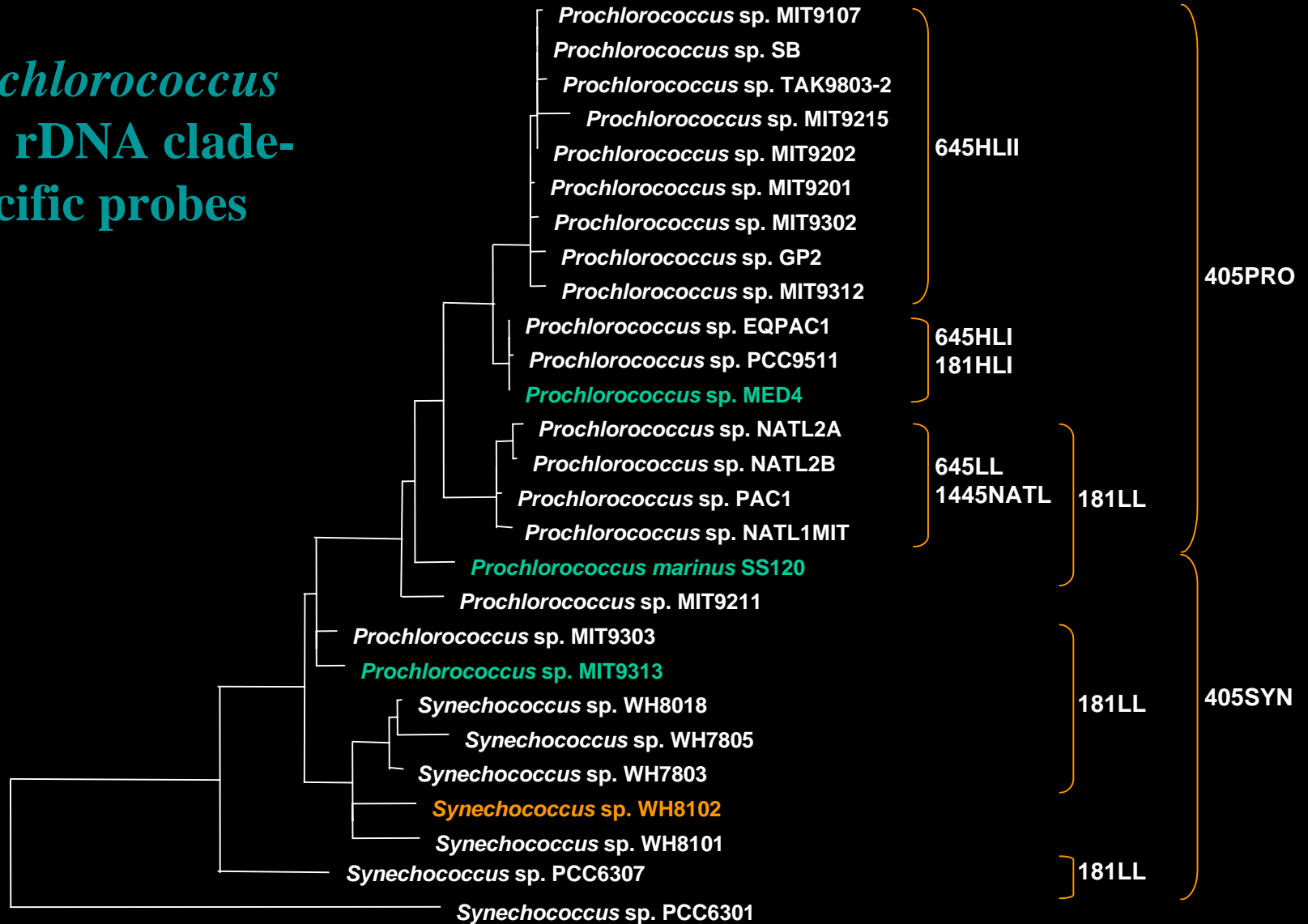


thermocline

nutrients

upwelling

Prochlorococcus 16S rDNA clade- specific probes



0.1

TABLE 1 Oligonucleotide primers and probes used in dot blots and DGGE analysis

PCR Primers	Specific target DNA	Sequence 5'-3'
OXY107F*	Oxygenic phototroph biased	GGA CGG GTG AGT AAC GC GTG R
OXY1313R*	Oxygenic phototroph biased	CTT CAC GTA GGC GAG TTG CAG C
CYA359F†	Oxygenic phototroph specific	GGG GAA TYT TCC GCA ATG GG
PRO1017R	<i>Prochlorococcus</i> , excluding MIT9303, MIT9313, MIT9302	TCC CGA AGG CAC CCT CWA AA
27F	Eubacterial	AGA GTT TGA TCM TGG CTC AG
1224R	Eubacterial	CAT TGT AGC ACG TGT GTA
Oligonucleotide Probes	Specific target DNA	Sequence 5'-3'
S1PRO634R	HLI <i>Prochlorococcus</i>	GCC GAT CAG TTT CCA CTG
S2PRO634R	HLII <i>Prochlorococcus</i>	GCC TTT CAG TTT CCA CTG
DPRO634R	LL <i>Prochlorococcus</i> (except SS120)	GCC AAT CAG TTT CCA CTG
SARG634R	SS120	GCC CTT CAG TTT CCA CTG
MIT1023R	MIT9303	TGC GTT CCC AAA GGC ACT
EUB338	Eubacteria	GCT GCC TCC CGT AGG AGT
PRO444R ‡	<i>Prochlorococcus</i> , excluding MIT9303, MIT9313	TAT TCC TCA AGT ACC GTC ATA
MAR572R	Marine picophytoplankton clade biased	GCC GCC TGC GGA CGC TTT

† A GC-clamp consisting of a 40 nucleotide GC-rich sequence (5'-CGC CCG CCG CGC CCC GCG CCG GTC CCG CCG CCC CCG CCC-3') was attached to the 5' end of the primer and used in DGGE analysis

‡ Based on SAR6R

TABLE 2 Oligonucleotide primers and probes used in FISH*

Oligonucleotide Probes	Specific target DNA	Sequence 5'-3'
S1PRO634R	HLI <i>Prochlorococcus</i>	GCC GAT CAG TTT CCA CTG
645 HLI*	HLI <i>Prochlorococcus</i>	ACC ATA CTC AAG CCG ATC
S2PRO634R	HLII <i>Prochlorococcus</i>	GCC TTT CAG TTT CCA CTG
645 HLII*	HLII <i>Prochlorococcus</i>	ACC ATA CTC AAG CCT TTC
DPRO634R	LL <i>Prochlorococcus</i> (PAC1/NATL1-MIT cluster)	GCC AAT CAG TTT CCA CTG
645 LL*	LL <i>Prochlorococcus</i> (PAC1/NATL1-MIT cluster)	ACC ATA CTC AAG CCA ATC
181 HLI*	HLI <i>Prochlorococcus</i>	CAT TTC ACC TAT CGG CAT
181 LL*	LL <i>Prochlorococcus</i> (not MIT9211 & includes some Syns)	CAT TTC ACC TCT CGG CAT
148 LL-MIT*	LL <i>Prochlorococcus</i> (MIT9211, MIT9303, MIT9313 only)	CCG TTT CCA ACC GTT ATC
405PRO*	Gen <i>Prochlorococcus</i> (General, but not MIT9303, MIT9313)	AGA GGC CTT CGT CCC TCA

Fluorescent in situ hybridisation (FISH)

PROBE DESIGN

Comparative sequence analysis

oligonucleotide

Monolabeled-fluorochrome
Fluorescein, Rhodamine, CY3,
BODIPY

C6 amino-link

HRP-labelled

Samples on filters or glass slides

Samples on filters or glass slides

46°C ↓ ↓ 35°C

Hybridisation

48°C ↓ ↓ 35°C

washing

equilibration

Incubation with
fluorochrome/tyramide

washing

HYBRIDISATION

Observe under fluorescence microscope

Prochlorococcus PCC9511 hybridised with HLI probe + TSA



HLI +ve

HLI and LL dual stain

HLI and WH8103 mix
HLI +ve

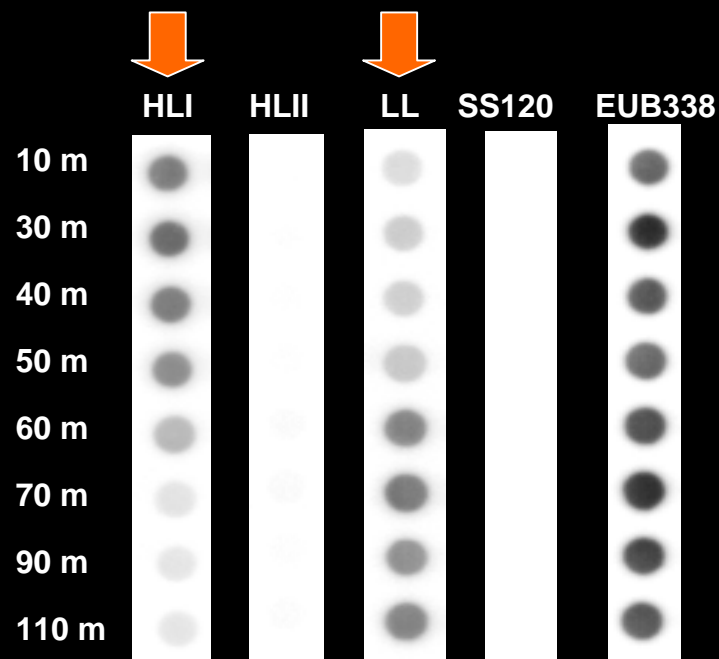
WH8103 -ve

Specificity of
16S rDNA
oligonucleotide
probes
for use in FISH

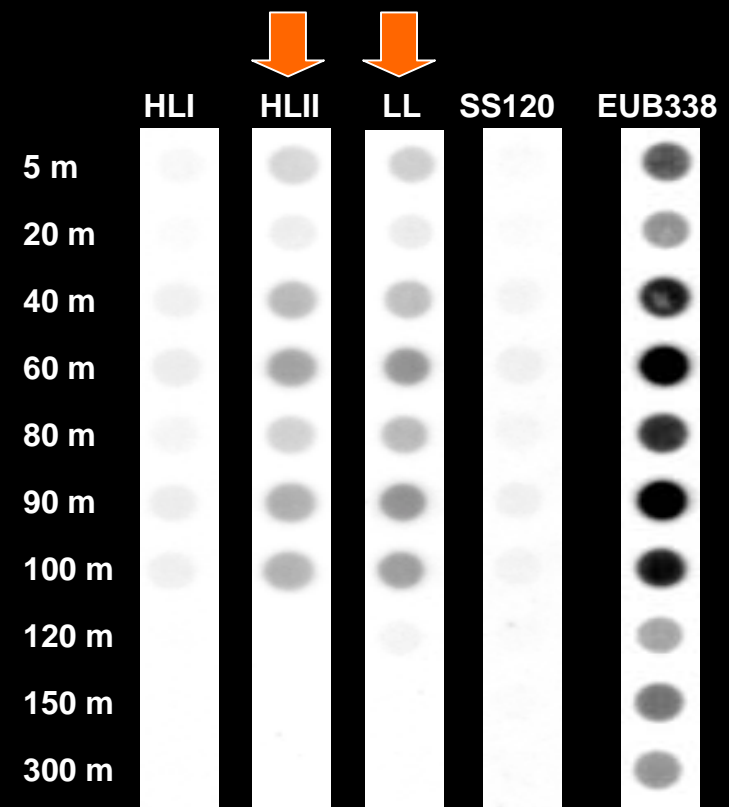
WH8103 +ve

Geographical and vertical distribution of *Prochlorococcus*

Eastern North Atlantic*



Sargasso Sea**



* stratified or ** mixed water column

FISH analysis of natural *Prochlorococcus* populations I

- North Atlantic
 - positive signals with HLI and LL

Depth (m) Proportion of DAPI stained cells giving a signal with each probe (%)

	645HLI	181LL	CYA664
3	22	<1	23
40	20	<1	23
80	0	13	14

Photophysiological characteristics of *Prochlorococcus* isolates grown at low irradiance ($9 \mu\text{mol Q m}^{-2} \text{s}^{-1}$) : relation to primary productivity estimates

This distribution of multiple *Prochlorococcus* ecotypes in the same water column would result in greater integrated production than could be achieved by a single ecotype

e.g. estimates of 1° production for the same number of cells in the low light of the deep photic zone (irradiance = $10 \mu\text{mol Q m}^{-2} \text{s}^{-1}$) using the strain specific values of $a^*_{\text{chl}a}$, ϕ_{max} and $\text{chl}a_2$ per cell, then the contribution of low-light adapted MIT9313 to 1° production would be 3.4 x higher than its high-light adapted co-isolate MIT9312

$a^*_{\text{chl}a}$ = spectrally weighted averaged Chl a2-specific absorption coefficient

ϕ_{max} = quantum yield (ratio of $\alpha_{\text{chl}a}$ to $a^*_{\text{chl}a}$)

From Moore *et al.*, (1998) Nature 393: 464-467

The Gulf of Aqaba system

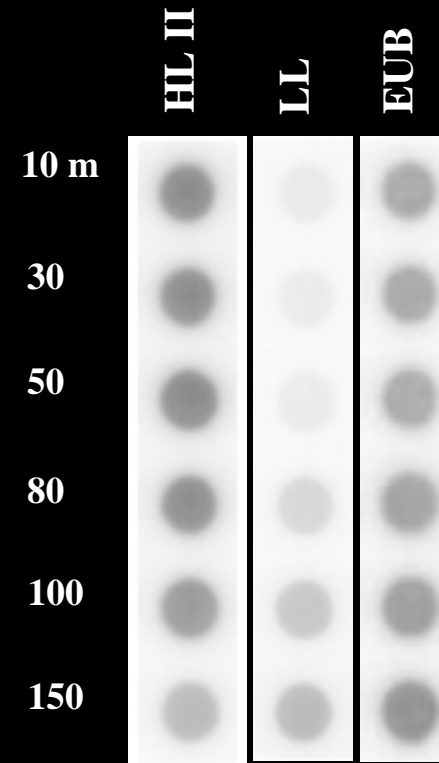
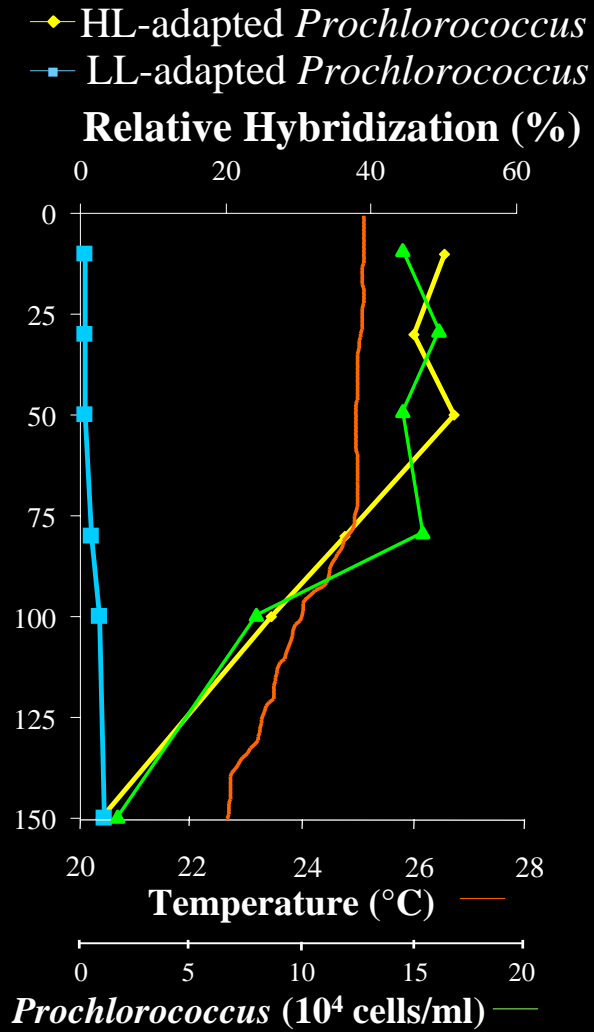


- Section of rift valley, up to 1800 m deep
 - Shallow southern sill (250 m)
 - ACW circulation
 - Residence time: surface = 1 yr; deep = 3-8 yr
 - Contains Red Sea surface water
 - No rivers
 - Low biomass & chl *a* → deep light penetration
 - Characteristic of open ocean, cf. southern Red Sea
 - Strong seasonal changes
 - Seasonal succession of picophytoplankton
- } oligotrophic



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Prochlorococcus niche partitioning - HLII dominance in the Red Sea



FISH analysis of natural *Prochlorococcus* populations II

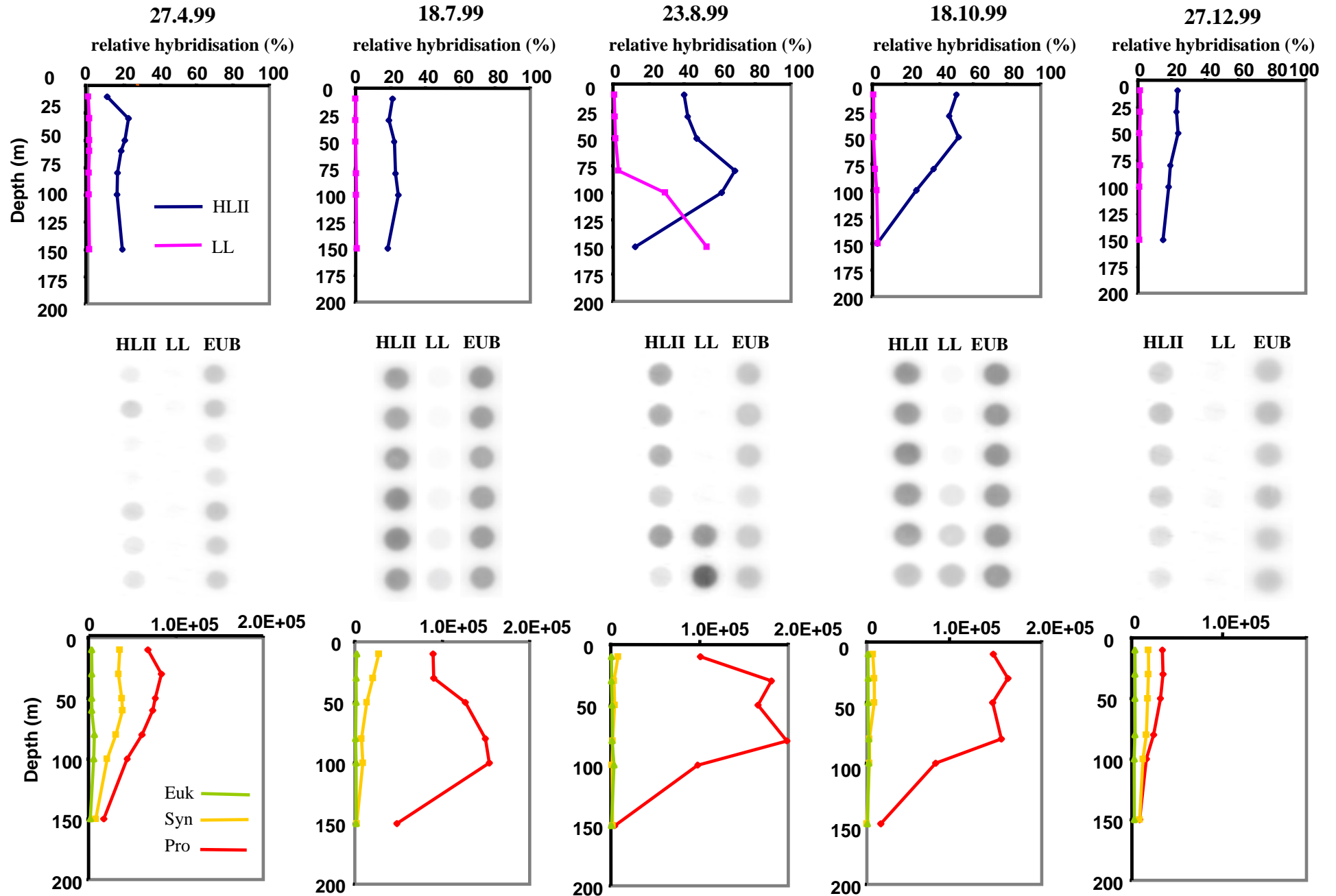
- Red Sea
 - positive signals with HLII and LL

Depth (m)

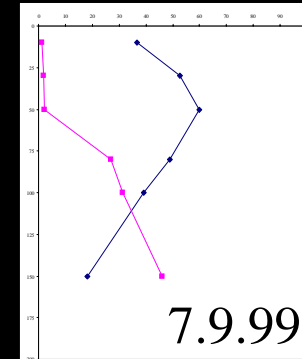
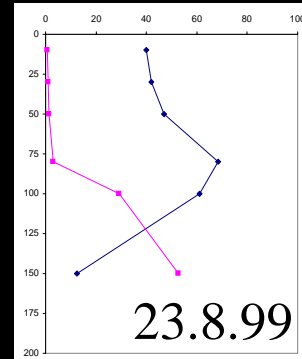
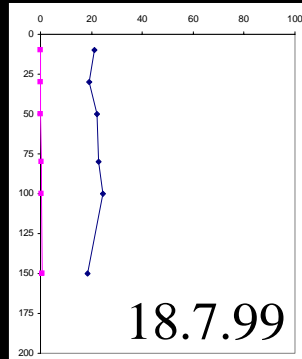
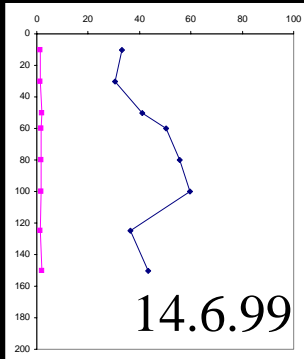
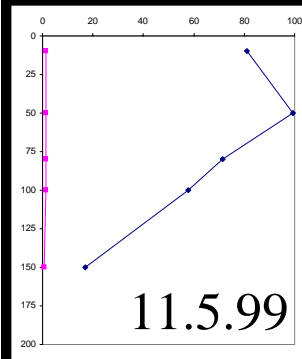
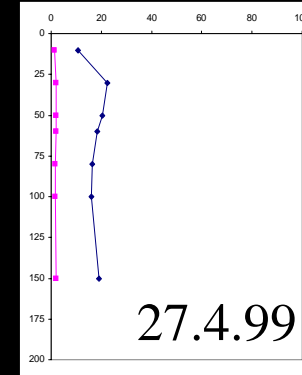
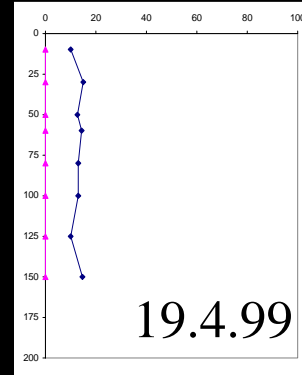
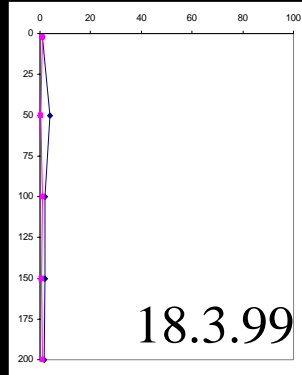
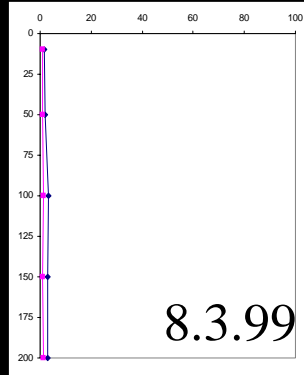
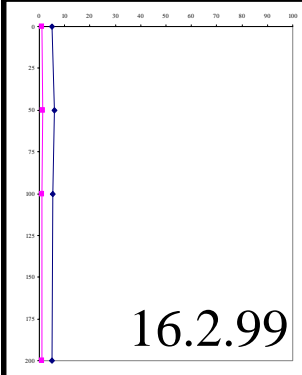
**Proportion of DAPI stained cells giving
a signal with each probe (%)**

	645HLII	181LL	1445NATL	405PRO	405SYN	CYA664
10	11.8	0.2	0.1	15.8	0.2	16.8
50	8.1	0.1	0.1	18.5	0.7	17.0
100	9.9	2.0	2.0	15.8	0.2	17.1

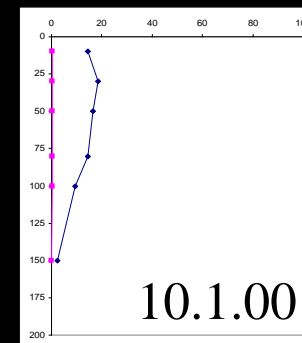
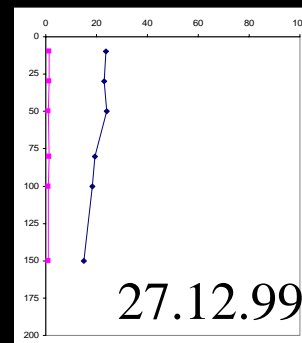
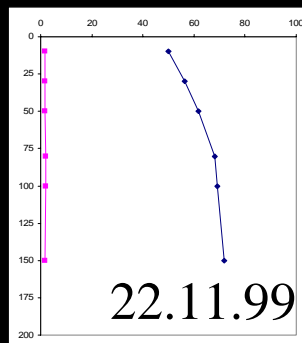
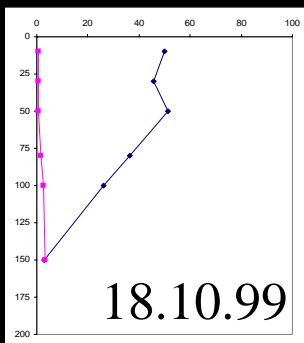
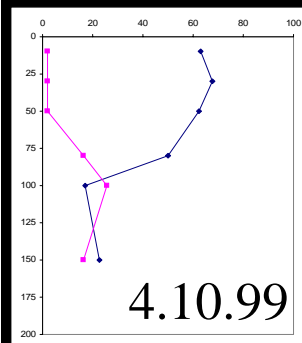
Prochlorococcus population dynamics Red Sea



Prochlorococcus



— LL
— HLII



Comparison of physiological properties of *Prochlorococcus* strains MED4 (HLI), TAK9803 (HLII) and SS120 (LL)

	MED4	TAK9803	SS120
<i>chl_b</i> / <i>a₂</i> ratio	low (0.05 -0.15)	low	high (0.4 -2.4)
optimal growth irradiance	15-80*	?	8-30 **
major antenna apoproteins	~ 32.5 kDa	~33 kDa	28-34 kDa
copies of <i>pcb</i> gene	single	single	multiple (7)
phycoerythrin	absent	absent	present
P inducible protein	present	absent	absent
growth on nitrate	no	no (?)	no

* photoinhibited only around 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

** photoinhibited at light intensities greater than 37 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

N.B. MED4 and SS120 genomes appear to be co-linear; 16S rDNA identity = 98.3%

Prochlorococcus Genome data**

http://www.jgi.doe.gov/JGI_microbial/html/index.html

Strain	Ecotype	Genome size	% GC	total genes	no. of genes unique to each strain
MED4	high-light adapted	1.66 Mb	30	1700	200 e.g a DNA photolyase (repairs UV damage)
MIT9313	low-light adapted	2.40 Mb	50	2300	550 e.g. ~ 20 transporters* & membrane proteins

* MIT9313 can utilise both ammonium and nitrite but MED4 only ammonium as a N source - consistent with genetic information; % 16S rDNA similarity = 97.4%

** data from Hess *et al.*, 2001 Photosyn. Res. & A. Dufresne and D. Vaultot, pers. comm.

1. *Synechococcus* Probes

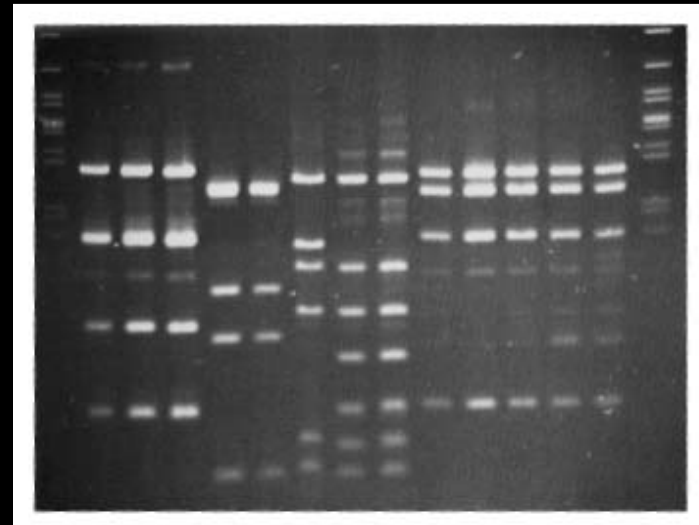
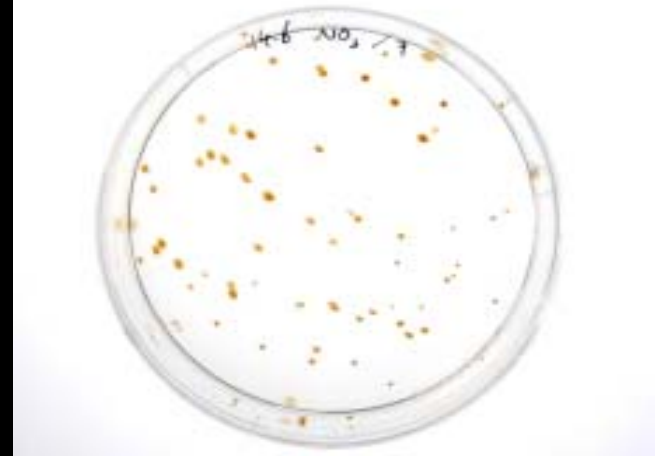
Strain isolation (250)



Screening isolates (*ntcA* RFLP)

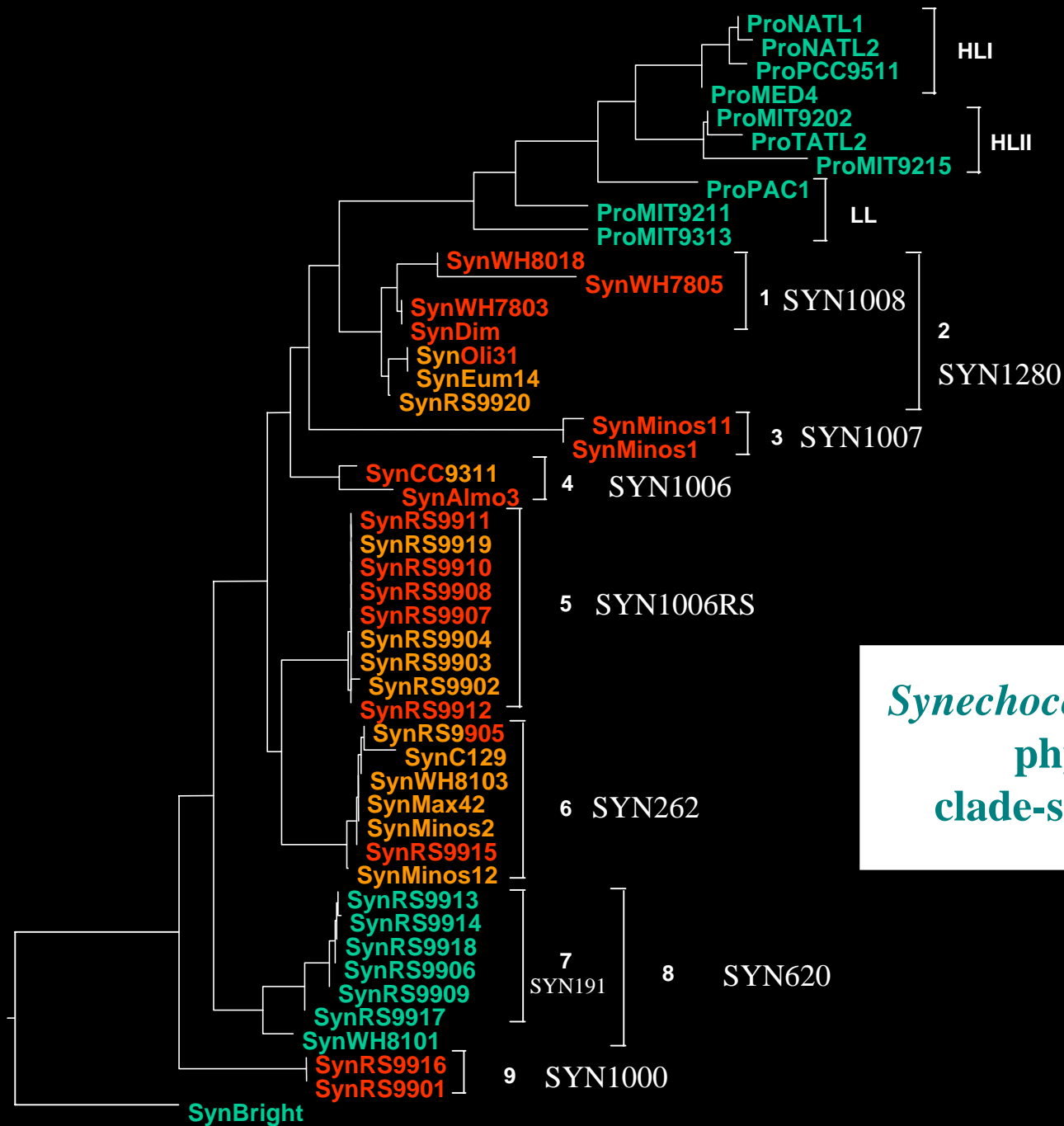


6 RFLP types; 20 clonal *Synechococcus* strains



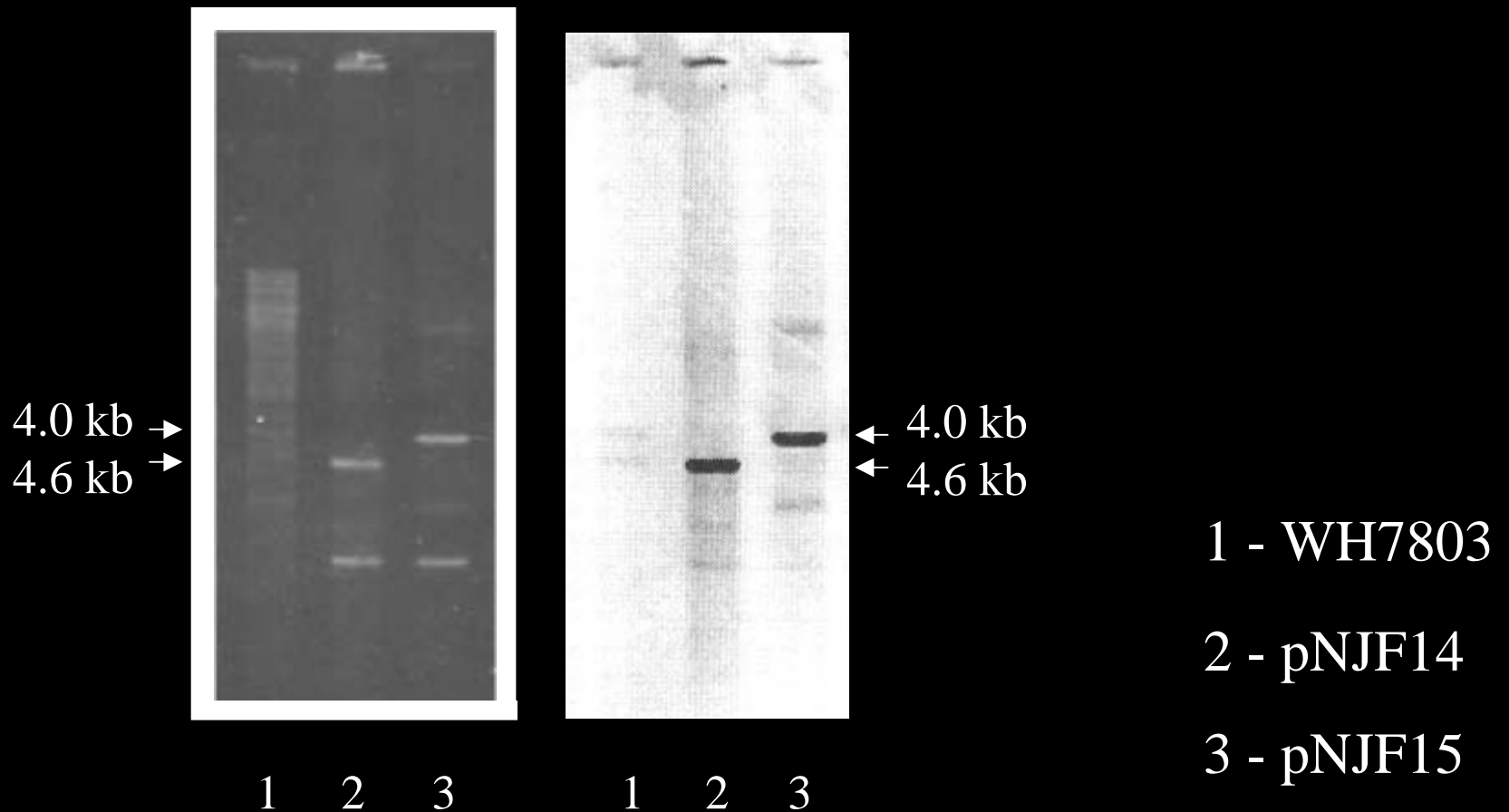
Analysis of Red Sea *Synechococcus* strains

Strain	Date of isolation	Depth of isolation	Colour	PUB/PEB ratio	Motility	N source	<i>ntcA</i> RFLP type	16S sequence
RS9901	29.3.99	0 m	Red	0.7	-	NO ₃ ⁻ /NH ₄ ⁺	I	+
RS9902	29.3.99	0 m	Orange	1.4	-	NO ₃ ⁻ /NH ₄ ⁺	II	+
RS9903	11.5.99	10 m	Orange	2.0	-	NO ₃ ⁻ /NH ₄ ⁺	III	+
RS9904	14.6.99	10 m	Orange	1.5	-	NO ₃ ⁻ /NH ₄ ⁺	III	+
RS9905	18.7.99	10 m	Orange/Red	1.0	-	NO ₃ ⁻ /NH ₄ ⁺	IV	+
RS9906	23.8.99	10 m	Green	-	-	NO ₃ ⁻ /NH ₄ ⁺	N/A	+
RS9907	23.8.99	10 m	Red	0.5	-	NO ₃ ⁻ /NH ₄ ⁺	II	+
RS9908	7.9.99	10 m	Red	0.5	-	NO ₃ ⁻ /NH ₄ ⁺	II	+
RS9909	7.9.99	10 m	Green	-	-	NO ₃ ⁻ /NH ₄ ⁺	N/A	+
RS9910	7.9.99	10 m	Red	0.5	-	NO ₃ ⁻ /NH ₄ ⁺	II	+
RS9911	11.5.99	10 m	Red	0.8	-	NH ₄ ⁺	V	+
RS9912	23.8.99	10 m	Red	0.5	-	NH ₄ ⁺	N/A	+
RS9913	7.9.99	10 m	Green	-	-	NH ₄ ⁺	N/A	+
RS9914	18.10.99	10 m	Green	-	-	NH ₄ ⁺	N/A	+
RS9915	18.10.99	10 m	Red	0.8	-	NH ₄ ⁺	IV	+
RS9916	22.11.99	10 m	Red	0.7	-	NH ₄ ⁺	I	+
RS9917	22.11.99	10 m	Green	-	-	NH ₄ ⁺	N/A	+
RS9918	22.11.99	10 m	Green	-	-	NH ₄ ⁺	N/A	+
RS9919	22.11.99	50 m	Orange	1.8	-	NO ₃ ⁻	V	+
RS9920	22.11.99	150 m	Orange	1.7	-	NO ₃ ⁻	VI	+



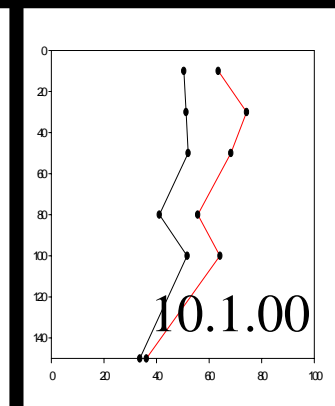
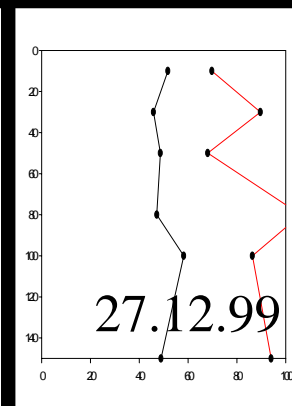
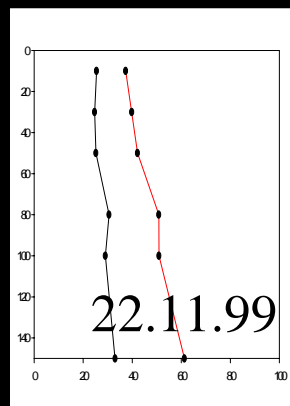
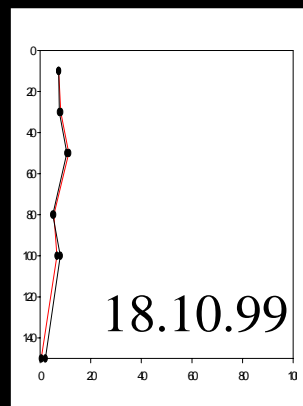
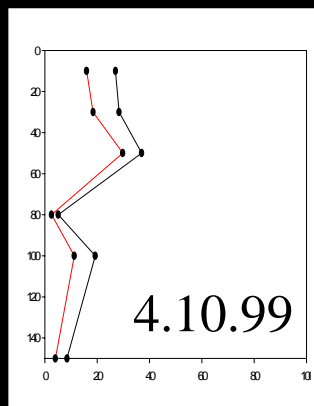
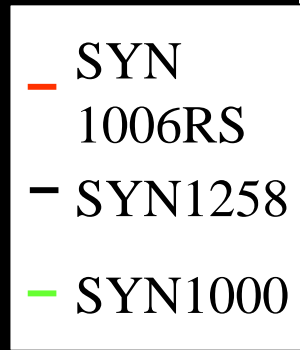
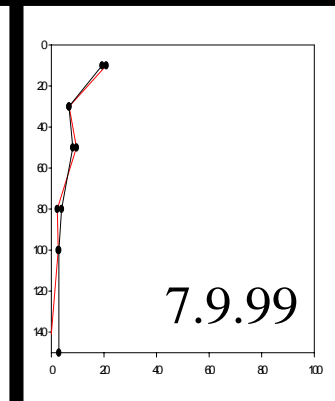
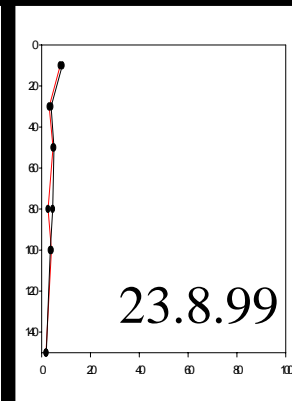
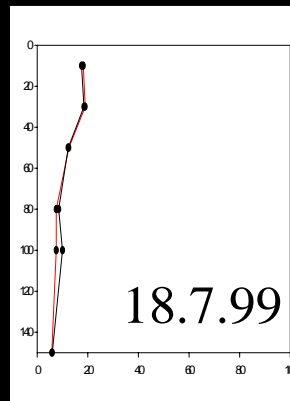
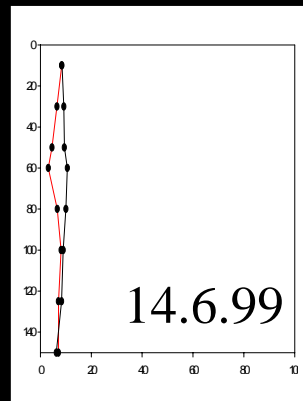
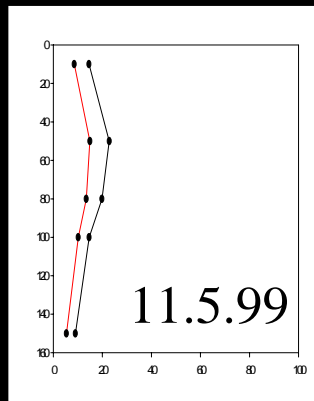
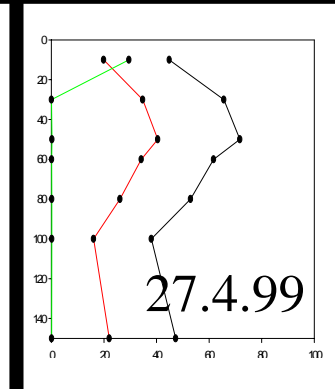
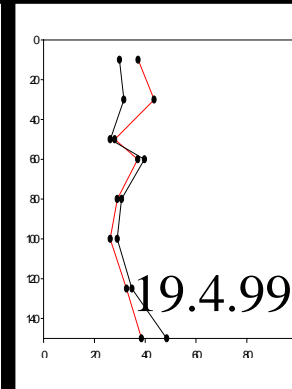
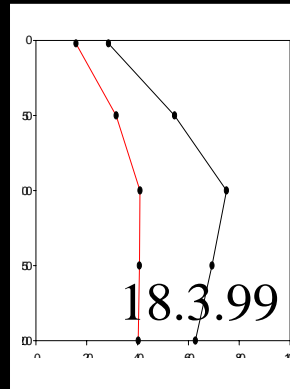
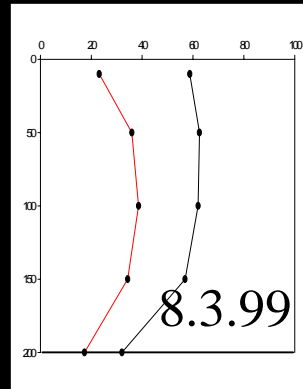
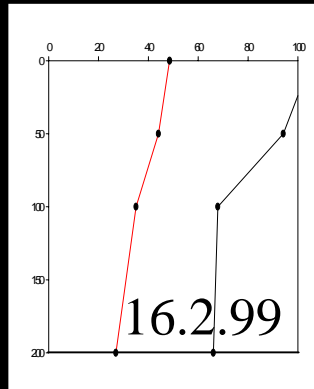
Synechococcus 16S rDNA
 phylogeny &
 clade-specific probes

Multiple 16S *rrn* copies



- Sequence identity between copies = 100%

Synechococcus



General comparison of marine cyanobacterial genomes

Organism	Genome Size (Mb)*	%GC*	Total no. of genes*	No. of genes unique to that genome#
<i>Prochlorococcus</i> MED4	1.67	30.9	1696	216
<i>Prochlorococcus</i> MIT9313	2.40	50.7	2195	554
<i>Synechococcus</i> WH8102	2.42	59.5	2426	536

* based on annotation from the JGI

A. Dufresne and D. Vaulot, pers. comm.

Comparison of cyanobacterial genomes with specific respect to complexity of two-component systems and ABC transporters

Organism	Histidine kinases [#]	Response regulators [#]	ABC nutrient transport systems [*]
<i>Prochlorococcus</i> MED4	6	7	25
<i>Prochlorococcus</i> MIT9313	7	12	42
<i>Synechococcus</i> WH8102	6	14	42
<i>Synechocystis</i> sp. PCC6803	44	52	54
<i>Nostoc punctiforme</i>	146	102	94

[#] based on annotation from the JGI / Cyanobase

^{*} based on the no. of genes encoding the ATPase component from JGI / Cyanobase annotation

Conclusions

- Distinct partitioning of *Prochlorococcus* genotypes occurs *in situ* and even within the same ecotype dramatic differences in community structure occur
 - this has implications for primary production estimates
- The marine *Synechococcus* genus contains much microdiversity but the exact shape & size of the niches occupied by specific strains remains to be elucidated as do the molecular and biochemical mechanisms of niche adaptation
- Phytoplankton dynamics in the Red Sea appear to be markedly influenced by the degree of deep winter mixing, and thus their initial seeding environment
 - *Prochlorococcus* in the Red Sea shows a domination by HLII genotypes, and LL genotypes adapted to a niche deep in the euphotic zone. There is marked seasonal variation in abundance, possibly reflecting water currents in the Gulf
- *Synechococcus* dynamics appear to be more complicated, yet show year-round domination by a single genotype
 - Readily culturable *Synechococcus* strains are not necessarily the dominant ones in the water column

Do different *Synechococcus* genotypes have different C-fixation capabilities?
How to address this *in situ*?

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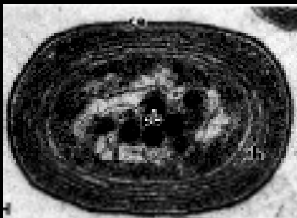
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***Synechococcus* strains**

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